

The spaA gene cloning of SE-9 strain by using PCR primer designed from the spaA gene sequence (SEQ ID#1) of Fujisawa strain

SEQ ID NO:3: Sense primer designed for preparation of SpaA and Δ SpaA protein by PCR amplification

Sequence length: 37 base pairs, which corresponds to the sequence of from the 79th to 106th nucleotide residues in SEQ ID NO:1

SEQ ID NO:4: Antisense primer designed for preparation of Δ SpaA protein by PCR amplification

Sequence length: 33 base pairs, which corresponds to the sequence of from the 1240th to 1260th nucleotide residues in SEQ ID NO:1

SEQ ID NO:5: Antisense primer designed for preparation of SpaA protein by PCR amplification

Sequence length: 38 base pairs, which corresponds to the sequence of from the 1855th to 1881st nucleotide residues in SEQ ID NO:1

SEQ ID NO:7: *Erysipelothrix rhusiopathiae* spaA gene, partial cds, strain:SE-9

Sequence length: 1748 base pairs, which corresponds to the sequence of from the 107th to 1854th nucleotide residues in SEQ ID NO:1

Expression of Spa A protein of SE-9 strain

First line; SEQ ID NO:1 Nucleotide position number

Second line; SEQ ID NO:2

→ atg aaa aag aaa aaa cac cta ttt ccg aaa gta agt ctt atg tcg tgc 48

→ Met Lys Lys Lys Lys His Leu Phe Pro Lys Val Ser Leu Met Ser Cys

→ xxx xxx xxx xxx xxx xxx xxx xxx xxx xxx xxx xxx xxx xxx xxx

→ *** **

→ 1 5 10 15

Third line; SEQ ID NO:7, SEQ ID NO:3, Antisense SEQ ID NO:5

Fourth line; Translated amino acid sequence from SEQ ID NO:7

Amino acid position number (in bold)

The spaA gene cloning of SE-9 strain by using PCR primer designed from the spaA gene sequence (SEQ ID#1) of Fujisawa strain

Expression of Spa A protein of SE-9 strain

1
atg aaa aag aaa aaa cac cta ttt ccg aaa gta agt ctt atg tcg tgc 48
Met Lys Lys Lys Lys His Leu Phe Pro Lys Val Ser Leu Met Ser Cys
xxx xxx xxx xxx xxx xxx xxx xxx xxx xxx xxx xxx xxx xxx xxx
*** *** *** *** *** *** *** *** *** *** *** *** *** *** *** ***

tta ctt tta aca gca atg cca cta caa aca gct ttt gct gat tcg aca 96
Leu Leu Leu Thr Ala Met Pro Leu Gln Thr Ala Phe Ala Asp Ser Thr
70 79
|→ (NcoI site) Sequence ID#3
xxx xxx xxx xxx xxx xxx xxx cat gcc atg gct ttc gct gat tcg aca
*** *** *** *** *** *** *** *** *** Met Ala Phe Ala Asp Ser Thr
20 25 30

gat att tct gtg att cca cta atc ggt gaa caa gtt gga ttg ctc cca 144
Asp Ile Ser Val ~~Ile Phe Leu Thr Gly Gln Gln Val Gly Leu Leu Phe~~
107
→ | → Sequence ID#7
gat att tct gtg att cca cta atc ggt gaa caa gtt gga ttg ctc cca
Asp Ile Ser Val ~~Ile Phe Leu Thr Gly Gln Gln Val Gly Leu Leu Phe~~
35 40 45

gtt tta cct ggg aca ggg gta cat gct cag gaa tac aac aaa atg act 192
~~Val Leu Phe Gly Thr Gly~~ Val ~~Ile Ala Gln Gln Tyr Asp Lys Ser Thr~~
gtt tta cct ggg aca ggg ata cat gct cag gaa tac aac aaa atg act
~~Val Leu Phe Gly Thr Gly~~ Ile ~~Ile Ala Gln Gln Tyr Asp Lys Ser Thr~~
50 55 60

The spaA gene cloning of SE-9 strain by using PCR primer designed from the spaA gene sequence (SEQ ID#1) of Fujisawa strain

gat gct tat att gaa aaa ttg gta tct cta att aat caa aaa gtg aag 240

Asp Ala Tyr Ile Glu Lys Leu Val Ser Leu Ile Asn Gln Lys Val Lys

206(a→g)

gat gct tat att gaa aat ttg gta tct cta att aat caa aaa gtg aag

Asp Ala Tyr Ile Glu Asn Leu Val Ser Leu Ile Asn Gln Lys Val Lys

Gly

65

70

75

80

ccg ttt ctt ata aat gag cca aag ggg tac caa agt ttc gaa gca gtg 288

Pro Phe Leu Ile Asn Gln Pro Tyr Gln Tyr Gln Ser Phe Gln Ala Val

ccg ttt ctt ata aat gaa cca aag ggg tac caa agt ttc gaa gca gtg

Pro Phe Leu Ile Asn Gln Pro Lys Gln Tyr Gln Ser Phe Gln Ala Val

85

90

95

aat gaa gag att aac tcg att gta agt gaa ctt aaa aat gaa gga atg 336

Asn Gln Gln Ile Asn Ser Ile Val Ser Gln Leu Lys Asn Gln Gln Met

aat gaa gag att aac tcg att gta agt gaa ctt aaa cat gaa gga atg

Asn Gln Gln Ile Asn Ser Ile Val Ser Gln Leu Lys His Gln Gln Met

100

105

110

agt ctt caa aac att cac cat atg ttt aaa caa agc atc caa aac cta 384

Ser Leu Gln Asn Ile His His Met Phe Lys Gln Ser Ile Gln Asn Leu

agt ctt caa aac att cac cat atg ttt aaa caa agc atc caa aac cta

Ser Leu Gln Asn Ile His His Met Phe Lys Gln Ser Ile Gln Asn Leu

115

120

125

gca act aga atc ggc tac aga agt ttt atg cag gat gct atg tat ctt 432

Gln His Arg Ile Gly Tyr Arg Ser Phe Met Gln Asn Ala Met Tyr Leu

gca act aga atc ggc tac aga agt ttt atg cag gat gct atg tat ctt

Gln Thr Arg Ile Gly Tyr Arg Ser Phe Met Gln Asn Ala Met Tyr Leu

130

135

140

The spaA gene cloning of SE-9 strain by using PCR primer designed from the spaA gene sequence (SEQ ID#1) of Fujisawa strain

gaa aat ttt gaa aga tta acg att cct gaa ctt gat gaa gca tac gtt 480

gaa Arg Phe Glu Arg Leu Thr Ile Pro Glu Leu Asp Glu Ala Tyr Val

461(a→g)

gaa aat ttt gaa aga tta acg att cct gaa ctt gat gaa gca tac gtt

gaa Arg Phe Glu Arg Leu Thr Ile Pro Glu Leu Asp Glu Ala Tyr Val

Gly

145

150

155

160

gat tta ctc gtg aat tac gag gtg aaa cac cgt att tta gta aaa tat 528

asp Leu Leu Val Asp Tyr Glu Val Lys His Arg Ile Leu Val Lys Pro

gat tta ctc gtg aat tac gag gtg aaa cac cgt att tta gta aaa tat

asp Leu Leu Val Asp Tyr Glu Val Lys His Arg Ile Leu Val Lys Pro

165

170

175

gaa ggt aaa gtt aaa ggt aga gct ccc tta gaa gca ttt ata gtt cct 576

gaa Gly Lys Val Lys Glu Arg Ala Pro Leu Glu Ala Phe Ile Val Pro

gaa gat aaa gtt aaa ggt aga gct cca tta gaa gca ttt ata gtt cct

gaa Asp Lys Val Lys Glu Arg Ala Pro Leu Glu Ala Phe Ile Val Pro

180

185

190

cta aga gat aga att cgt agt atg aat gaa att gct gca gaa gta aat 624

leu Arg Asp Arg Ile Arg Ser Met Asp Glu Ile Ala Ala Glu Val Asp

608(t→c)

cta aga aat aga att cgt agt atg aat gaa att gct gca gaa gta aat

leu Arg Asn Arg Ile Arg Ser Met Asp Glu Ile Ala Ala Glu Val Asp

Thr

195

200

205

The spaA gene cloning of SE-9 strain by using PCR primer designed from the spaA gene sequence (SEQ ID#1) of Fujisawa strain

tat tta cct gaa gcg cat gag gat ttc tta gtt tca gat tca agc gag 672

Tyr Leu Pro Gln Ala His Gln Asp Phe Leu Val Ser Asp Ser Ser Gln

642(t→g)

tat tta cct gaa gcg cat gag gat ttc tta gtt tca gat tca agc gag

Tyr Leu Pro Gln Ala His Gln Asp Phe Leu Val Ser Asp Ser Ser Gln

Gln

210

215

220

tat aat gac aaa cta aat aat atc aac ttt gct ttg ggt cta ggg gtc 720

Tyr Asp Asp Lys Leu Asp Asp Ile Asn Phe Ala Leu Gly Leu Gly Val

tat aat gac aaa cta aat aat atc aac ttt gct ttg ggt cta ggg gtc

Tyr Asp Asp Lys Leu Asp Asp Ile Asn Phe Ala Leu Gly Leu Gly Val

225

230

235

240

agc gag ttt att gac tat aac cgg ctc gaa aat atg atg gaa aaa gaa 768

Ser Gln Phe Ile Asp Tyr Asn Arg Leu Gln Asn Met Met Gln Lys Gln

758(t→c)

agc gag ttt att gac tat aac cgg ctc gaa aat atg atg gaa aaa gaa

Ser Gln Phe Ile Asp Tyr Asn Arg Leu Gln Asn Met Met Gln Lys Gln

Thr

245

250

255

ctt cat cca ctg tat ctt gaa ctt tat gct atg cgg aga aat cgc caa 816

Leu His Pro Leu Tyr Leu Gln Leu Tyr Ala Met Arg Arg Asn Arg Gln

att cat cca ttg tat ctt gaa ctt tat gct atg cgg aga aat cgc caa

Ile His Pro Leu Tyr Leu Gln Leu Tyr Ala Met Arg Arg Asn Arg Gln

260

265

270

The spaA gene cloning of SE-9 strain by using PCR primer designed from the spaA gene sequence (SEQ ID#1) of Fujisawa strain

att caa gtt gta aga gat gta tat cca aac ttg gaa cgt gcg aac gcg 864

Ile Glu Val Val Arg Asp Val Tyr Pro Asp Leu Glu Arg Ala Asp Ala

333(a→g)

att caa gtt gta aga gat gta tat cca aac ttg gaa cgt gcg aac gcg

Ile Glu Val Val Arg Asp Val Tyr Pro Asp Leu Glu Arg Ala Asp Ala

Gly

275

280

285

gtt gtt gaa tcc tta aag aca att aaa gat ata aaa caa aga ggg aag 912

Val Val Glu Ser Leu Lys Thr Thr Lys Asp Thr Lys Glu Arg Gly

gtt gtt gaa tcc tta aag aca att aaa gat ata aaa caa aga gag aag

Val Val Glu Ser Leu Lys Thr Thr Lys Asp Thr Lys Glu Arg Glu

290

295

300

aaa cta cag gaa ctt ctt gaa att tat atc caa aga agt gga gat gtt 960

Asp Leu Glu Glu Leu Leu Glu Thr Tyr Thr Glu Arg Ser Gly Asp Val

aaa cta cag gaa ctt ctt gaa att tat atc caa aga agt gga gat gtt

Lys Leu Glu Glu Leu Leu Glu Thr Tyr Thr Glu Arg Ser Gly Asp Val

305

310

315

320

cga aaa cca gat gta ctc caa cga ttt att gga aaa tat caa tca gta 1008

Arg Lys Pro Asp Val Leu Glu Arg Phe Thr Glu Lys Tyr Glu Ser Val

cga aaa cca gat gta ctc caa cga ttt att gga aaa tat caa tca gta

Arg Lys Pro Asp Val Leu Glu Arg Phe Thr Glu Lys Tyr Glu Ser Val

325

330

335

gtt gat gaa gaa aaa aat aaa ctt caa gat tat tta gaa tca gat att 1056

Val Asp Glu Glu Lys Asp Lys Leu Glu Asp Tyr Leu Glu Ser Asp Thr

gtt gat gaa gaa aaa aat aaa ctt caa gat tat tta gaa tca gat att

Val Asp Glu Glu Lys Asp Lys Leu Glu Asp Tyr Leu Glu Ser Asp Thr

340

345

350

The spaA gene cloning of SE-9 strain by using PCR primer designed from the spaA gene sequence (SEQ ID#1) of Fujisawa strain

ttt gat tca tat agt gtg gat ggc gag aaa ata aga aat aaa gaa att 1104
the Asp ser Lys ser Val Asp Glu Glu Lys Ile Arg Asp Lys Glu Ile
ttt gat tca tat agt gtg gat ggc gag aaa ata aga aat aaa gaa att
the Asp ser Lys ser Val Asp Glu Glu Lys Ile Arg Asp Lys Glu Ile
355 360 365

aca ctc atc aat aga gat gca tac tta tct atg att tac aga gct caa 1152
the Leu Ile Asp Arg Asp Ala Tyr Leu ser Met Ile Tyr Arg Ala Glu
aca ctc atc aat aga gat gca tac tta tct atg att tac aga gct caa
the Leu Ile Asp Arg Asp Ala Tyr Leu Ser Met Ile Tyr Arg Ala Glu
370 375 380

tcg att tcg gaa att aag acg att cgt gca gat tta gaa tca ctt gtc 1200
ser Ile Ser Glu Ile Lys Thr Ile Arg Ala Asp Leu Glu Ser Leu Val
tcg att tcg gaa att aag acg att cgt gca gat tta gaa tca ctt gtc
ser Ile Ser Glu Ile Lys Thr Ile Arg Ala Asp Leu Glu Ser Leu Val
385 390 395 400

1240
| ← Antisense
cct gaa agt

aaa tca ttc caa aat gaa gaa agt gac tct aaa gta gag cct gaa agt 1248
Lys Ser Phe Glu Asp Glu Glu Tyr Asp Ser Lys Val Glu Pro Glu Ser
aaa tca ttc caa aat gaa gaa agt gat tct aaa gta gag cct gaa agt
Lys Ser Phe Glu Asp Glu Glu Tyr Asp Ser Lys Val Glu Pro Glu Ser
405 410 415

The spaA gene cloning of SE-9 strain by using PCR primer designed from the spaA gene sequence (SEQ ID#1) of Fujisawa strain

1260

Sequence ID#4 | (BamHISite)

ccc gtt aaa gta taa gga tcc gcg

Stop

ccc gtt aaa gta gaa aaa cca gtt gat gaa gaa aaa cct aaa gat caa 1296

Pro Val Leu Val Gln Pro Pro Val Asp Glu Ala Lys Pro Lys Asp Gln

ccc gtt aaa gta gaa aaa cca gtt gat aaa gaa aaa cct aaa gat caa

Pro Val Lys Val Gln Lys Pro Val Asp Lys Ala Lys Pro Lys Asp Gln

420 425 430

aag aag cta gtt gat caa tca aaa ccc gaa tgc aat tca aaa gaa ggg 1344

Lys Lys Leu Val Asp Gln Ser Lys Pro Gln Ser Asp Ser Lys Gln Gln

aag aag cca gtt gat caa tca aaa ccc gaa tgc aat tca aaa gaa ggg

Lys Lys Pro Val Asp Gln Ser Lys Pro Gln Ser Asp Ser Lys Gln Gln

435 440 445

tgg att aag aaa gat aat aag tgg ttc tat att gag aaa tca ggt gga 1392

Trp Thr Lys Lys Asp Asp Lys Trp Phe Tyr Thr Gln Lys Ser Gly Gly

tgg att aag aaa gat aat aag tgg ttc tat att gag aaa tca ggt gga

Trp Thr Lys Lys Asp Asp Lys Trp Phe Tyr Thr Gln Lys Ser Gly Gly

450 455 460

atg gca aca ggt tgg aag aag gta gca gac aaa tgg tac tac ctc gat 1440

Met Ala Thr Gly Trp Lys Lys Val Ala Asp Lys Trp Tyr Tyr Leu Asp

atg gca aca gga tgg aag aag gta gga gac aaa tgg tac tac ctc gat

Met Ala Thr Gly Trp Lys Lys Val Gly Asp Lys Trp Tyr Tyr Leu Asp

465 470 475 480

The spaA gene cloning of SE-9 strain by using PCR primer designed from the spaA gene sequence (SEQ ID#1) of Fujisawa strain

```

aat acg ggt gct ata gtt acg ggt tgg aag aag gta gca aac aaa tgg      1488
Leu Thr Gly Ala Ile Val Thr Gly Trp Lys Lys Val Ala Asp Lys Trp
aat acg ggt gct atg gtt acg ggt tgg aag aag gta gca aac aaa tgg
Leu Thr Gly Ala Met Val Thr Gly Trp Lys Lys Val Ala Asp Lys Trp
                                485                490                495

tac tat ctt gaa aaa tca ggt gcg atg gca aca gga tgg aag aaa gta      1536
Leu Thr Leu Glu Lys Ser Gly Ala Met Ala Thr Gly Trp Lys Lys Val
tac tac ctt gaa aac tca ggt gcg atg gca aca gga tgg aag aaa gta
Leu Thr Leu Glu Asn Ser Gly Ala Met Ala Thr Gly Trp Lys Lys Val
                                500                505                510

tca aac aag tgg tac tac ctt gaa aac tca ggt gca atg gca aca gga      1584
Ser Asp Lys Trp Tyr Tyr Leu Glu Asn Ser Gly Ala Met Ala Thr Gly
tca aac aag tgg tac tac ctt gaa aac tca ggt gcg atg gca aca gga
Ser Asp Lys Trp Tyr Tyr Leu Glu Asn Ser Gly Ala Met Ala Thr Gly
                                515                520                525

tgg aag aaa gta tca aac aag tgg tac tac ctt gaa aat tca ggc gca      1632
Trp Lys Lys Val Ser Asp Lys Trp Tyr Tyr Tyr Leu Glu Asn Ser Gly Ala
1591(a→g)
tgg aag aga gta tca aac aag tgg tac tac ctt gaa aat tca ggc gca
Trp Lys Arg Val Ser Asp Lys Trp Tyr Tyr Tyr Leu Glu Asn Ser Gly Ala
Gly
                                530                535                540

atg gct aca gga tgg aaa aag gta gca aac aaa tgg tac tac ctt gaa      1680
Met Ala Thr Gly Trp Lys Lys Val Ala Asp Lys Trp Tyr Tyr Tyr Leu Glu
atg gct aca gga tgg aaa aag gta gca aac aaa tgg tac tac ctt gaa
Met Ala Thr Gly Trp Lys Lys Val Ala Asp Lys Trp Tyr Tyr Tyr Leu Glu
                                545                550                555                560

```

The spaA gene cloning of SE-9 strain by using PCR primer designed from the spaA gene sequence (SEQ ID#1) of Fujisawa strain

aac tca ggt gcg atg gca aca gga tgg aag aaa gta tcg aac aag tgg 1728

Asn Ser Gly Ala Met Ala Thr Gln Trp Lys Lys Val Ser Asn Lys Trp

aac tca ggt gcg atg gca aca gga tgg aag aaa gta tcg aac aag tgg

Asn Ser Gly Ala Met Ala Thr Gln Trp Lys Lys Val Ser Asn Lys Trp

565

570

575

tac tac ctt gaa aac tca ggc gca atg gct aca gga tgg aaa aag gta 1776

Tyr Tyr Leu Glu Asp Ser Gly Ala Met Ala Thr Gly Trp Lys Lys Val

tac tac ctt gaa aac tca ggc gca atg gca acg ggt tgg aag aaa ata

Tyr Tyr Leu Glu Asp Ser Gly Ala Met Ala Thr Gly Trp Lys Lys Ile

580

585

590

gca aac aaa tgg tac tac ctt gat aaa tca gga atg atg gtt aca ggt 1824

Ala Asn Lys Trp Tyr Tyr Leu Asp Lys Ser Gly Met Met Val Thr Gly

gca aat aaa tgg tac tac ctt gat aaa tca gga atg atg gtt aca ggt

Ala Asn Lys Trp Tyr Tyr Leu Asp Lys Ser Gly Met Met Val Thr Gly

595

600

605

tca aaa tct att gat ggt aaa aag tat gca ttt aag aac gat gga agt 1872

Ser Lys Ser Thr Asp Gly Lys Lys Thr Ala

Phe Lys Asn Asp Gly Ser

1855

Sequence ID#7

→

|

← Antisense Sequence ID#5

tca aaa tct att gat ggt aaa aag tat gca ttt aag aac gat gga agt

Ser Lys Ser Thr Asp Gly Lys Lys Thr Ala

Phe Lys Asn Asp Gly Ser

610

615

620

tta aaa tag 1881

Leu Lys Stop

1831

| (BamHISite)

tta aaa tag agg gat ccg cg

Leu Lys Stop

625

630